Fig. 1 The fisherman and his catch. The giant squid was hooked in a 5 m deep small bay. The squid was dying at the time of capture. Total length was about 10 m, the tentacles were about 7.3 m, and the weight about 220 kg (photograph: J. M. Lillebøe, B.T., Norway).

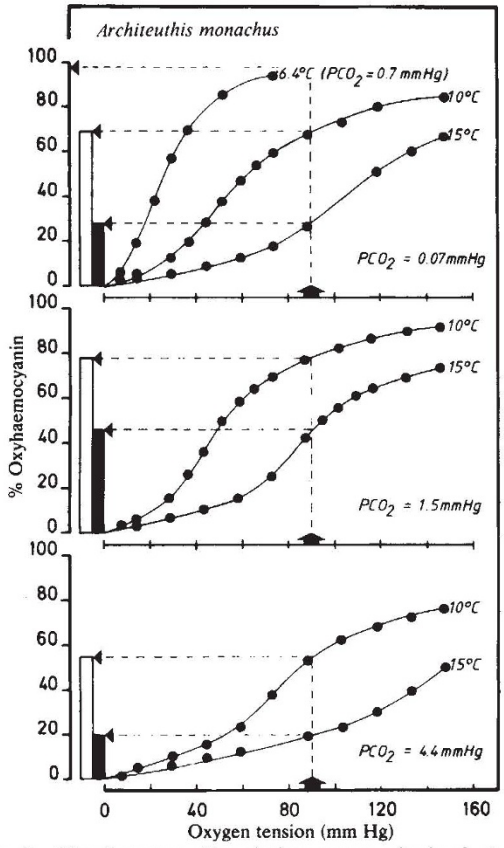

Fig. 2 Blood oxygen dissociation curves obtained at constant P_{CO_2}s of 0.07 mm Hg (pH = 7.93), 1.5 mm Hg (pH = 7.60), and 4.4 mm Hg (pH = 7.30) at 10 and 15 °C. P_{CO_2} was 0.7 mm Hg (pH = 7.73) at 6.4 °C. The large arrows indicate the typical cephalopod arterial P_{O_2} of 90 mm Hg[7]. The bars show estimated arterial O_2 saturations at 10 °C (open bar), and 15 °C (filled bar) assessed from the O_2 equilibrium curves measured for *A. monachus* blood.

would thus be less than half loaded with O_2 at 15 °C and a P_{CO_2} of 1.5 mm Hg, whereas at 10 °C the pigment would still be about 80% saturated (Fig. 2).

Increased activity may raise blood P_{CO_2}. At a P_{CO_2} of 4.4 mm Hg the O_2 saturation would decrease to about 55% at 10 °C, and it may be that the O_2 requirement could not be satisfied at this temperature taking into account the very low O_2 carrying capacity of the haemocyanin (0.3 mM O_2). At 15 °C less than one-fifth of the haemocyanin would be loaded with O_2. Even a relatively small increase in ambient temperature may thus have a very serious effect on tissue O_2 provision in the giant squid due to the pronounced temperature sensitivity of its blood.

Hyperventilation decreasing the general level of blood P_{CO_2} will normally favour O_2 loading in the gills of cephalopods because of the high pH sensitivity of their blood (Bohr shift)[7–9]. *A. monachus* blood as shown here, however, has a reversed Bohr shift below a P_{CO_2} of 0.7 mm Hg, a feature not previously recorded for cephalopod blood (Fig. 2). Hyperventilation and a respiratory alkalosis would thus further impair O_2 loading.

The high temperature sensitivity of the O_2 affinity in combination with the very low O_2 carrying capacity of the blood could thus be a cause of death for giant squids experiencing higher than normal water temperatures along the shores of Newfoundland, Great Britain and Scandinavia in the warm Atlantic current, which can in some years carry large volumes of warm water northwards.

The O_2 carrying capacity demonstrated in the present study for the giant squid is very low compared with the active oceanic cephalopods (1.6 to 1.9 mM O_2, ref. 10). This feature suggests that the giant squid is a relatively poor swimmer and a passive and sluggish predator[11].

I thank Rune Ystebö, who caught and took care of the squid and Professors Kjell Johansen and Hans Jörgen Fyhn and Drs Johanne Kjennerud and Gunnar Lykkeboe for helpful discussions, and Maria Sula Evjen for technical assistance.

Received 23 December 1982; accepted 24 March 1983.

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Growth of 'black smoker' bacteria at temperatures of at least 250 °C

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Complex communities of thermophilic bacteria have been cultured from the 350 °C waters emanating from sulphide chimneys, or 'black smokers', at 21 °N along the East Pacific Rise[1]. Several of the bacterial communities were shown to grow rapidly at 100 °C and atmospheric pressure, producing methane, hydrogen gas and carbon monoxide. These gases are found in superheated vent water, having previously been attributed to abiogenic reactions. Before concluding that these 'black smoker' bacteria actually contribute to the chemistry of the superheated hydrothermal fluids, it was necessary to test their ability to grow and produce gases at *in situ* vent tem-

0028–0836/83/220423—04$01.00

peratures and pressures. Here we report that a bacterial community originally cultured from 306 °C water is capable of chemolithotrophic growth in a titanium growth chamber under *in situ* vent pressure of 265 atm and at temperatures of at least 250 °C. (At 265 atm, seawater remains liquid at temperatures of at least 460 °C[2].) Transmission electron microscopy of thin sections of bacteria cultured at 250 °C has revealed the presence of at least two morphologically distinct organisms.

The upper temperature limit of environments known to support life has rarely been reported as exceeding 100 °C, although there have been reports of bacteria existing in hot waters at temperatures a few degrees above 100 °C[3-7], or cultured at 105 °C[7]. Only prokaryotic organisms are found in environments hotter than 75 °C, and at temperatures above 85 °C microbial diversity decreases sharply[3]. A stable microbial habitat at temperatures exceeding 100 °C requires liquid water, and therefore must be under either hydrostatic or osmotic pressure. Such environments exist on Earth, but none are so dramatic as the deep-sea hydrothermal systems recently discovered along submarine tectonic rifts and ridges[8,9]. One of the vent types found along the East Pacific Rise, and at the Gorda Ridge and the Guaymas Basin, is characterized by conical sulphide chimneys ('smokers') out of which spew superheated waters at temperatures exceeding 350 °C[8-10]. These waters contain supersaturated levels of reduced gases and metals[8,11-13], potential energy sources for bacteria which serve as the primary producers in the vent food chains[8,14]. They also contain complex thermophilic microbial communities capable of rapid growth and gas production at 100 °C and atmospheric pressure[1]. We were prompted to incubate one of the intact communities at even higher temperatures and pressure to determine whether the bacteria were capable of growth in *in situ* vent conditions (350 °C and 265 atm).

The apparatus used to monitor the microbial community at high temperature and pressure, without cooling or decompressing the primary culture, was a modification of an earlier design described by Yayanos[15] (see Fig. 1 legend). The bacterial content of subsamples was estimated by epifluorescent microscopy, after staining with the DNA-specific dye 4',6'-diamidino-2-phenylindole 2 HCl (DAPI). Protein concentrations in duplicate subsamples were determined by both the Folin–Lowry and Coomassie blue methods.

Growth curves of these mixed cultures at 150, 200, 250 and 300 °C are shown in Fig. 1. Bacterial doubling times were 8 h at 150 °C, 1.5 h at 200 °C and 40 min at 250 °C. At 250 °C the protein concentration doubled at the same rate as the number of bacteria. Incubation at 265 atm clearly expanded the temperature range for growth, and required at least 250 °C for optimum growth rates equivalent to those at 100 °C and atmospheric pressure. At 300 °C, an apparent short growth spurt was followed by a decline in the number of single bacteria (Fig. 1*d*). During this decline clumps of a few to several hundred cells began to appear, and membranous spheres ranging in size from less than 1 to 10 μm occurred singly or in clumps of 20 or more (see Fig. 2*d*). DAPI-stainable particles were associated with these spheres. Concomitant with the appearance of clumps and membranous spheres was an increase in the level of particulate protein, which continued throughout the course of the experiment. Concentrations of soluble DNA, ranging from less than 1 μg to 5 μg DNA per ml, were also detected in the medium during this time. One possible interpretation of these data is that bacteria continue to reproduce at 300 °C, as shown by the microscopic observations and increase in protein concentrations, but that lysis occurred during the decompression required to collect the sample releasing DNA. Some lysis may also have occurred at 265 atm as a result of marked decrease in *p*H which is known to occur in seawater at 300 °C under pressure[16]. An uninoculated medium control incubated at 200 °C for 16 h contained no DAPI-stainable particles. When mercury was introduced into an inoculated control incubated in similar conditions, the number of DAPI-stainable cells decreased at a rate of approximately 1 log unit per 30 min (Fig. 1*b*).

The amino acid composition of total protein extracted from cells in late log growth phase at 250 °C is shown in Table 1, primarily to substantiate that the colorimetric methods were measuring complete protein. Almost all of the amino acids were detected, although there were five unknown amino acid and/or peptide peaks that represented over 25% of the total protein. The unusually high levels of glycine, glutamic acid and serine in this protein preparation cannot presently be explained.

Electron micrographs of thin sections of the bacteria cultured at 250 °C illustrate the morphological diversity of this unique thermophilic microbial community. The bacteria shown in Fig. 2 were present in high numbers in late log and stationary growth stages at all temperatures examined. Most known bacteria that might be expected to grow in the extreme conditions of submarine hydrothermal vents are archaebacteria, and include the methanogens, *Thermoplasma* and *Sulfolobus*[3,17]. Although we did not observe organisms that lacked a cell wall, we noted other structures characteristic of some archaebacteria such as cavitations, internal membranes with subunits (Fig. 2*a*,*b*), and thick cell walls (Fig. 2*c*)[3,18-21]. The organisms in Fig. 2*c* also show a morphological resemblance to the thermophilic anaerobic microbe recently isolated from a shallow submarine solfatara environment and described by Stetter[7]. It is quite probable, however, that bacteria obtained from superheated vent waters are adapted for growth in the unusually extreme conditions found in submarine hydrothermal systems and thus possess unique structures, macromolecules and methods of replication.

The presence of methanogens in the inoculum we used for these high temperature/pressure growth studies was shown by initial experiments conducted at 100 °C and atmospheric pressure[1]. Some of the bacteria cultured at 250 °C and 265 atm autofluoresced at 420 nm, which is presumptive evidence of a coenzyme found only in methanogenic bacteria[22]. A gas sample from a 3 h culture at 300 °C contained 9.6% CH_4, 2.2% H_2 and 0.1% CO, none of which were detected in the uninoculated medium control at 200 °C.

These findings open up the possibility that bacteria may exist and grow within the Earth's crust at temperatures exceeding

Table 1 Amino acid analysis of total protein from a 6 h culture at 250 °C and 265 atm pressure

Amino acid	Amino acid residues ($\times 10^{-3}$)
Aspartic acid	78.0
Threonine	36.8
Serine	105.7
Glutamic acid	116.0
Proline	46.5
Glycine	332.5
Alanine	69.0
Half-cystine	5.5
Valine	25.9
Methionine	9.8
Isoleucine	24.0
Leucine	45.9
Tyrosine	4.7
Phenylalanine	20.2
Histidine	17.1
Lysine	23.6
Arginine	39.0

Bacterial cells from a 0.25 ml sample of the 250 °C culture were concentrated by centrifugation using a Beckman Microfuge B for 1 min. The protein was precipitated using 5% trichloroacetic acid as described for Fig. 2. The protein was dialysed in distilled water, lyophilized, and hydrolysed with 6.1 M HCl for 22 h at 110 °C in an evacuated sealed tube. The sample was analysed using a modified Beckman 120 B amino acid analyser. The sample contained approximately 200 μg protein as determined by colorimetric methods (see Fig. 1 legend). In addition to the amino acids listed, there were five unknown amino acid peaks. These unknown amino acids and/or peptides represented over 25% of the total protein analysed. Tryptophan, cysteine and glutamine could not be determined quantitatively by this method.

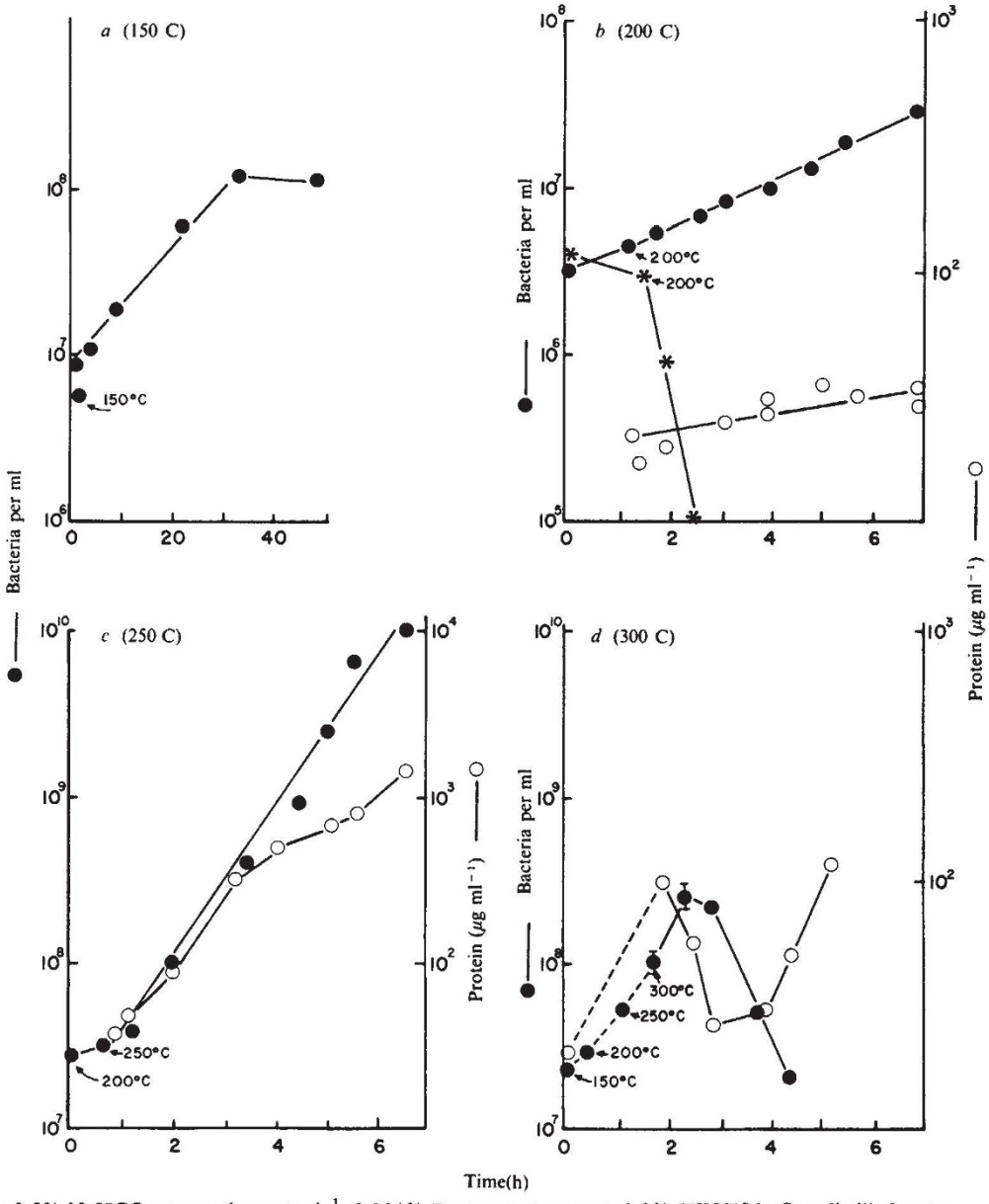

Fig. 1 Growth curves of a 'black smoker' bacterial community and associated increases in total protein at temperatures of 150 °C (*a*), 200 °C (*b*), 250 °C (*c*) and 300 °C (*d*), under hydrostatic pressure of 265 atm. In *b*, * shows a mercury (Hg)-killed control experiment. An uninoculated medium control was also incubated and sampled at 200 °C for a period of 16 h. During this time period no DAPI-stainable particles were observed in any of the collected samples. In the uninoculated control, as well as in all of the growth experiments, evidence of H_2S and SO_2 production was noted by odour. Arrows in *d* indicate bacterial counts at the times when specific temperatures were reached. Error bars on the initial and following point at 300 °C represent variations in counts from three different dilutions of each sample. Fifty fields were counted per sample dilution. The subsequent decrease in cell number was accompanied by an increase in the number of single and multiple membrane structures varying in size from less than 1 to 10 μm. These spheres contained small (0.2 μm) DAPI-stainable particles.

Methods: The culture was obtained originally from a 306 °C water sample collected at a depth of 2,650 m during the October–November 1979 RISE expedition to 21° N along the East Pacific Rise. Further details on the characteristics of the bacterial community along with culture and maintenance procedures have been described elsewhere[1]. The growth medium used was minimal salts[1] supplemented with 0.2% $Na_2S_2O_3 \cdot 5H_2O$; 0.02% $MnSO_4 \cdot H_2O$; 0.001% $FeSO_4 \cdot 7H_2O$; 0.03% $(NH_4)_2SO_4$; 0.001% $Ca(NO_3)_2$; 0.05% Na_2HPO_4; 0.5% $NaHCO_3$; trace element mix[1]; 0.001% Bacto-yeast extract; 0.3% HEPES buffer; distilled water, and adjusted to *p*H 6.5. The bacterial community was cultured at 100 °C in a water or sand bath for 48 h and diluted in fresh medium preheated to ~90 °C before each high temperature/pressure experiment. Bacterial cultures were loaded into a custom-made titanium syringe with a maximum volume of 120 ml. (A 50-ml glass syringe was used in the 150 °C growth experiment.) The syringe barrel is 20 cm long and 2.5 cm wide and consists of a titanium plunger fitted with a Teflon O-ring on the front end, and a Viton O-ring on both ends. The syringe was placed into a 750 ml pressure vessel containing high-temperature test silicon hydraulic fluid preheated to approximately 90–95 °C. The pressure vessel was entirely encased in a heating jacket. The syringe was fitted onto the valve cap of the pressure vessel. The syringe/pressure vessel/cap combination was then connected to pressure pumps and to the sample collecting vessel using high pressure-tested stainless steel tubing. Separate pressure pumps were used to apply equivalent pressures to the main culture pressure vessel and to the subsample pressure vessel. The sample fluid collected was at ambient temperature as a result of a temperature gradient in the stainless steel tubing. Before collecting a sample for analysis, 1.5–2 ml were transferred and discarded to ensure that residues of the previous samples were washed out of the stainless steel tubing. Approximately 2–4 ml of samples were usually collected for analysis. The cultures were collected in acid-cleaned test-tubes and immediately fixed with 2% (w/v) glutaraldehyde and maintained at 2–4 °C until counted. The pressure vessel was heated by a 140 V Variac, and the temperature regulated by F & M Model 220 power proportioning temperature controller. The temperature in the pressure vessel was measured by a thermistor-regulated controller as well as by a metal thermometer, both of which are inserted into the heating jacket. Generally, it took 35–39 min for a 50 °C increase within the range of 100–300 °C. The reaction vessel was pressurized to 265 atm before increasing the temperature, so as to avoid boiling the sample. The pressure was constantly monitored and adjusted during the temperature ascent period. When the desired temperature was reached, a time zero sample was taken. Total bacterial counts were measured by epifluorescent microscopy using the procedures of Porter and Feig[23]. Cells were stained with the DNA specific fluorescent dye 4′,6-diamidino-2-phenylindole 2HCl (DAPI). This dye binds specifically to adenine–thymine base pairs and has the advantage over acridine orange in that it does not interfere with other organic and inorganic compounds. Fixed cells were stained with DAPI to a final concentration of 0.02 μg ml^{-1} for 5 min. The stained cells were concentrated on 0.2 μm Nuclepore filters prestained with Irgalan black dye. Protein was extracted and measured using the procedures described by Gallant and Suskind[24]. Bacteria from two 0.5 ml samples were concentrated by centrifugation. Protein was precipitated in cold 5% trichloroacetic acid, solubilized in 1M NaOH, and measured by both the Folin–Lowry and Coomassie blue methods. For the Hg-killed control (*b*), 3 ml of Hg was pumped into the culture when the temperature reached 200 °C. Within 5 h there were fewer than 10^2 DAPI-stainable bacteria per ml in the sample. At the present time we cannot explain the mechanism of Hg disinfection at high temperature and high pressure, although it is quite likely that some Hg^{2+} ions are formed, as the medium in these conditions would be acidic[16]. We used this method for controls because a metallic Hg displacement vessel was used for collecting samples from the high temperature/high pressure growth chamber and so it was easy to transfer Hg into the culture.

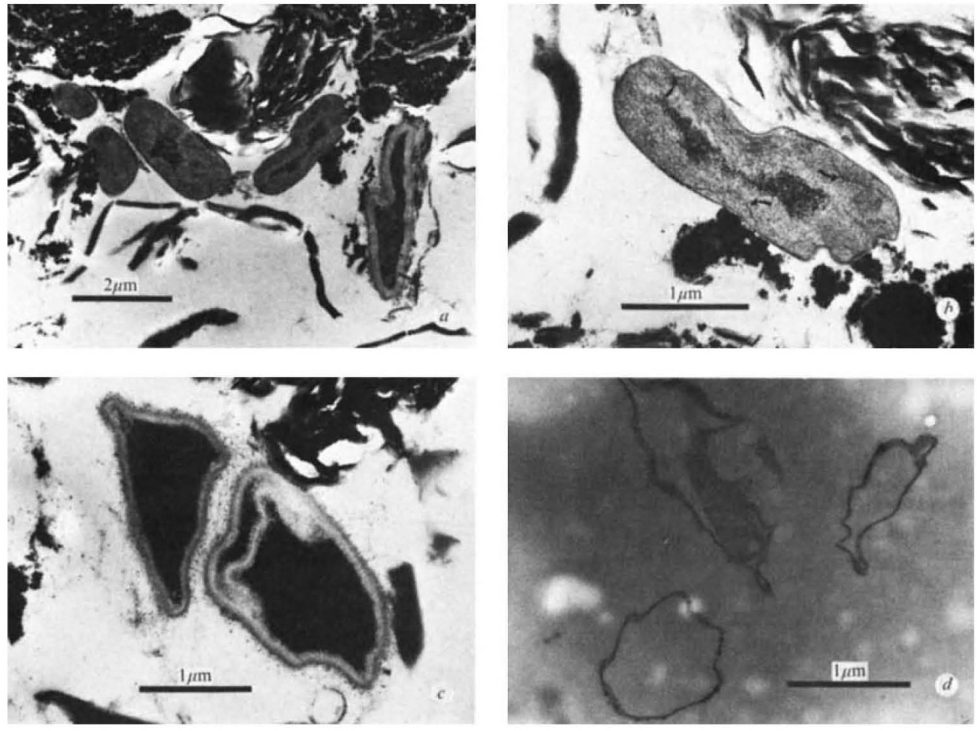

Fig. 2 Transmission electron micrographs of ultrathin sections of microorganisms commonly observed in the 150–300 °C samples. Samples were taken from a 7 h culture at 250 °C and 265 atm (*a–c*). The two most abundant rod-shaped bacteria are shown in *a*. Note the internal membranes (mesosomes) in the more abundant organisms, and the thick cell wall and lack of any definable internal structures in the bacterium on the right. These same two organisms are shown at higher magnification in *b* and *c*. Note the pleomorphism in both organisms. The internal wall–membrane complex seen in *c* is probably a result of sectioning through two planes of one organism. Vertical subunits are seen clearly in this internal cell wall–membrane complex. Thin sections of the spheres formed after 3 h at 300 °C are shown in *d*. The particulate protein measured in the 300 °C culture was derived from these spheres as observed microscopically. **Methods:** The cultures were subsampled from the pressure vessel and fixed immediately in glutaraldehyde (2.5% final concentration). The fixed cells were concentrated by centrifugation for 1 min. The pellet was resuspended in 0.2 M cacodylate buffer at *p*H 7.8. Cells were dehydrated in acetone, stained with 1% OsO_4 and 1% uranyl acetate, and sectioned.

250 °C. As the thermophilic bacterial community examined in this study has a wide range of physiological activities, including formation and utilization of oxidized and reduced metals, sulphur and trace gases, hypotheses related to the origin of these elements and compounds in submarine hydrothermal systems may need to be reassessed.

Finally, these results substantiate the hypothesis that microbial growth is limited not by temperature but by the existence of liquid water, assuming that all other conditions necessary for life are provided. This greatly increases the number of environments and conditions both on Earth and elsewhere in the Universe where life can exist.

This research was supported by the Biological Oceanography Division of the Office of Naval Research grant N00014-79C-0004, and by a NSF postdoctoral fellowship, PDF-8166007, awarded to J.W.D. through the Scripps Institution of Oceanography. We thank A. A. Yayanos, Ron Van Boxtel, Allan S. Dietz, C. Dahm, M. Lilley, S. Hoffman, M. de Angelis, L. Gordon, B. Becker, R. Y. Morita, A. Soeldner, C. Weiss and D. Lavole for their help.

Received 4 January; accepted 18 March 1983.

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Variable expression of Ia antigens on the vascular endothelium of mouse skin allografts

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Ia antigens are membrane-bound glycoproteins that play a part in antigen recognition and subsequent cell–cell interactions in the immune response. In the mouse they are coded for by the *I* region of the major histocompatibility complex *H–2* and have been demonstrated on B lymphocytes, monocytes, activated T cells, macrophages and dendritic cells, including Langerhans cells[1,2]. Ia-like antigens have also been detected on the vascular endothelium in man[3–6] and on epidermal keratinocytes in rats[7,8] but expression on the latter cells was induced by a graft-versus-host reaction or by contact hypersensitivity[9]. In the mouse, previous studies have suggested that Ia antigens in skin are restricted to epidermal Langerhans cells and it was thought that these were the targets for Ia-dependent rejection of skin allografts. The results presented here show that Ia antigens in mouse allografts are also present on the vascular endothelium but their expression is variable and dependent on the immunological status of the recipient. These findings suggest that vascular endothelial cells can act as targets in Ia-incompatible skin allograft rejection.

We investigated the presence of Ia antigens in mouse skin allografts and the localization of these antigens by *in vivo* injection of labelled anti-Ia alloantibodies. A monoclonal anti-Ia IgG2a alloantibody, raised in our laboratory in BALB/c ($H\text{–}2^d$) mice against BALB/K ($H\text{–}2^k$) donor cells, was used. The Ia^k determinant recognized by this antibody was shown to map in the *I* region by a positive binding reaction with 40–50%

0028-0836/83/220426—04$01.00